REVIEW

Plasticity in Development of the Central Nervous System

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ABSTRACT—Development of the central nervous system analyzed in quail-chick chimera is reviewed. The fate map of the brain vesicles has been studied. Posterior mesencephalon is shown to differentiate into the anterior cerebellum. Metencephalon differentiates into the posterior cerebellum. On the cytodifferentiation of the cerebellum, challenging results to the classical hypothesis have been obtained.

Heterotopic transplantations of the brain vesicles show that the brain vesicles have limited capacity to change their fate. Alar plate of the prosencephalon can differentiate into the optic tectum when transplanted into the mesencephalon. Rostral part of the mesencephalon has capacity to differentiate into the cerebellum, and rostral part of the metencephalon can differentiate into the optic tectum. Caudal part of the mesencephalon and metencephalon did not change their fate.

Rotation of the rostrocaudal axis of the tectum anlage at around 10 somite stage shows that rostrocaudal axis of the tectum is not determined at that stage. The rotated tectum is regulated of its rostrocaudal axis, and later cytoarchitectonic development and retinotectal map formation proceed according to the host axis. Rostrocaudal specificity of the optic tectum may be determined through interactions with surrounding tissue, and well organized retinotectal map may be achieved in a retinotopic manner.

INTRODUCTION

Most of the peripheral nervous system differentiate from the neural crest cells. Differentiation of the neural crest cells and plasticity of the peripheral nervous system have been studied well by quail-chick chimera system [1, 2]. The fate of the neural crest cells depends on the site from which they migrate. For example, parasympathetic cholinergic neurons of the gastrointestinal tract migrate from the level of the 1–7 somite, while sympathetic neurons migrate from the level caudal to the 7 somite. Neural crest cells from the level of 18–24 somite migrate into the adrenomedulla and differentiate into chromaffin cells. Heterotopic transplantations of the neural crest cells of the level of the 1–7 somite into that of the 18–24 somite showed that neural crest cells from the

transplant differentiated into the chromaffin cells of the adrenal medulla [3]. Recently it was shown by a sophisticated experiment that cholinergic neurons in ciliary ganglion can transdifferentiate into adrenergic cells when transplanted into adrenomedullary region [4].

Very recently, quail-chick chimera system has been applied to study development of the central nervous system (CNS), and here, the CNS development clarified by quail-chick chimera will be reviewed.

FATE MAP OF THE CNS

First, development of the CNS is summarized briefly [5]. Just after the closure of the neural tube at the cephalic level, three primitive brain vesicles are differentiated, that is, prosencephalon, mesencephalon and rhombencephalon. Telencephalon and diencephalon differentiate from the

prosencephalon, and finally differentiate into the cerebral hemisphere and the diencephalon, respectively. Optic tectum which is a main visual center of the lower vertebrates including birds, differentiates into mesencephalon. Rhombencephalon splits into metencephalon and myelencephalon. Cerebellum and pons differentiate from the metencephalon. Myelencephalon differentiates into the medulla oblongata.

Very recently interesting results on the fate of the brain vesicles were published from two laboratories. Homotopic trasplantations of the mesencephalon and metencephalon were performed by Hallonet *et al.* [6], and by Martinez and Alvarado-Mallart [7]. It was shown that the alar plate of the caudal part of the mesencephalon did not differentiate into the optic tectum but into the rostral part of the cerebellum. Metencephalon differentiated into the caudal part of the cerebellum. Purkinje cells are shown to differentiate after radial outward migration from the ventricular epithelium. Posterior mesencephalon did not produce external granular layer [6]. Hallonet *et al.* [6] examined the nuclear pattern and cell type at the anterior cerebellum, and found that cells in the molecular layer have the same nuclear marker as the ventricular epithelium not as the external granular layer. Hence, they suggested that cells in the molecular layer migrate from the ventricular epithelium not from the external granular layer. This suggestion is a challenge to the classical hypothesis that cells of the molecular layer migrate from the external granular layer.

PLASTICITY OF THE BRAIN VESICLES IN DIFFERENTIATION

Fate of brain vesicles after heterotopic transplantations has been tested [8–12].

Very interesting results have been obtained by Nakamura *et al.* [8, 9]. They transplanted the alar plate of the prosencephalon into the mesencephalon (Fig. 1), and found that the transplants differentiated the laminar pattern of the optic tectum when the transplants were integrated into the host (Fig. 2). As the optic tectum is a visual center in birds, it is an interesting question whether such optic tecta which differentiated from the prosencephalon receive inputs from the retina. To answer the question Nakamura *et al.* [9] used monoclonal antibody which specifically binds to chick neurofilament. They found that optic nerve fibers were continuous at the boundary of quail and chick domain. Retinal fibers ran in the stratum opticum of the tectum in a similar fashion both in the chick and quail domains. This result means
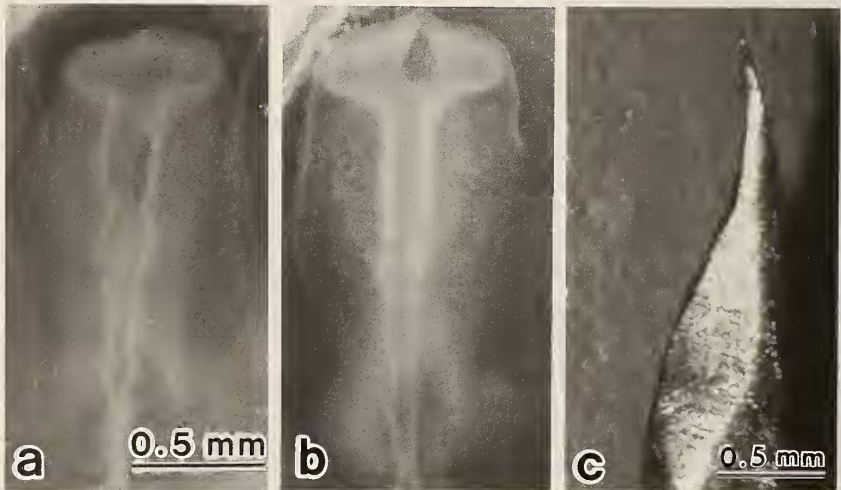

FIG. 1. A chick (a) and a quail (b) embryos at 9 somite stage, and a microsculpel (c). Dorsal part of the mesencephalon of the chick embryo is excised, and the embryo is ready for the graft. The quail embryo (b) is after the removal of a dorsal part of the prosencephalon. Transplatation is carried out with a microsculpel made of a steel needle.

that the optic tectum which differentiates from the prosencephalon can receive optic nerve fibers. Since the retinotectal relation is very strict, we are now testing whether such an optic tectum receives the fibers from the proper part of the retina or not.

Many of the prosencephalon which transplanted into the mesencephalon were not integrated into the host. At that time they did not differentiate into the optic tectum. These results suggest the importance of tissue interactions in the determina- tion of fate of the prosencephalon. Importance of tissue interactions in the CNS development is supported by the results of avian CNS develop- ment [12] and mammalian CNS development [13– 15]. Alvarado-Mallart et al. [12] transplanted mesencephalon into the prosencephalon. The transplanted mesencephalon differentiated into the optic tectum at the ectopic site. Since the host tissue participated in ectopic tectum formation, Alvarado-Mallart et al. suggested that the host
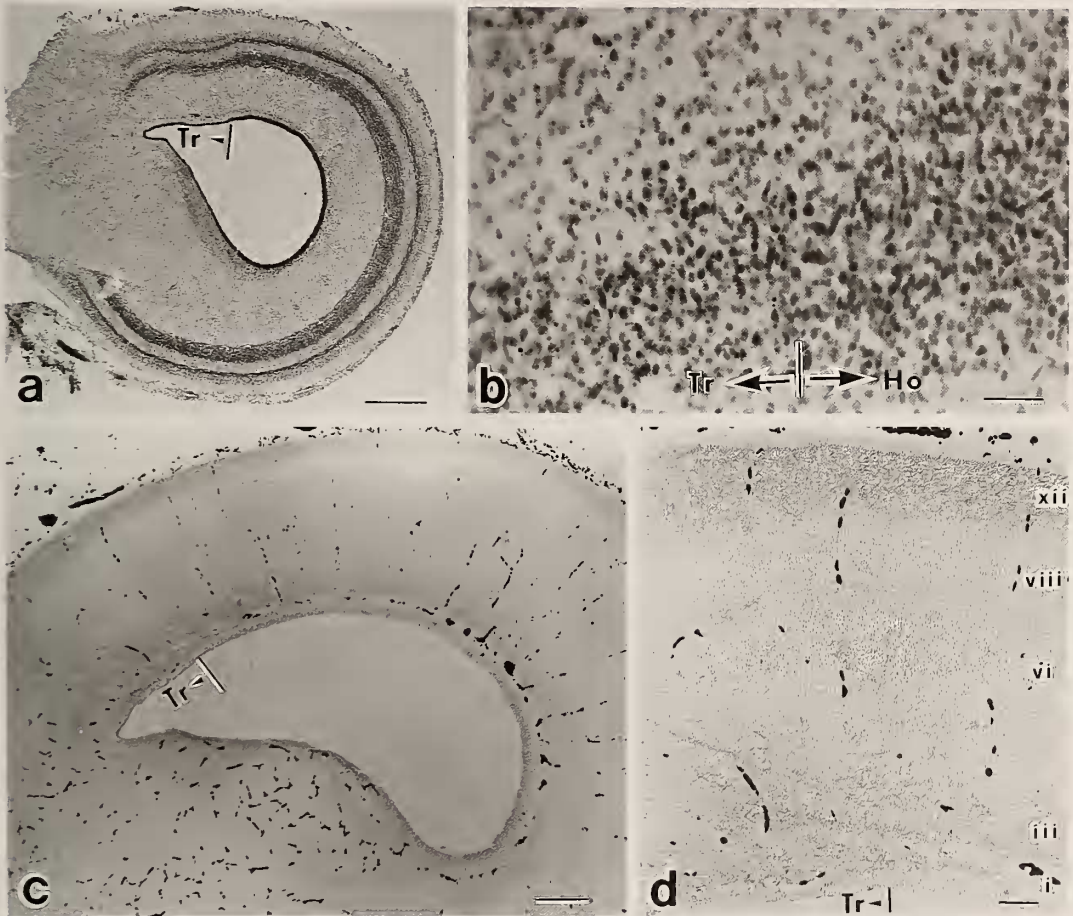

FIG. 2.   A chimeric tectum a part of which is differentiated from a dorsal part of the prosencephalon.
(a) Low magnification of a chimeric tectum. A prosencephalon transplant (Tr) differentiated as a part of the optic tectum. Bar: 500 μm.
(b) High magnification at the boundary of the transplant (Tr) and the host (Ho). Quail cells can be easily distinguished from chick cells because of the aggregation of heterochromatin after Feulgen-Rossenbeck staining. Bar: 25 μm.
(c), (d) Staining with the monoclonal antibody which binds specifically to the chick neurofilaments.
     Alternative sections were stained with Feulgen-Rossenbeck procedure and with monoclonal antibody which stains specifically chick neurofilaments. Optic nerve fibers run in a similar fashion both in the transplant and the host. (c) low magnification, Bar: 200 μm. (d) high magnification, Bar 50 μm. Tr: transplant. (Taken from Nakamura et al. [10])

prosencephalon near the transplant changed their fate and differentiated into the tectum after interaction with the transplant.

In rat embryos, it was shown that development of area-specific outputs is not a fixed property of cortical areas. This has been demonstrated by transplanting pieces of late fetal neocortex to heterotopic positions within the neocortex of newborn rats. The projection of the layer 5 of the transplant was dependent on the transplant's posi-
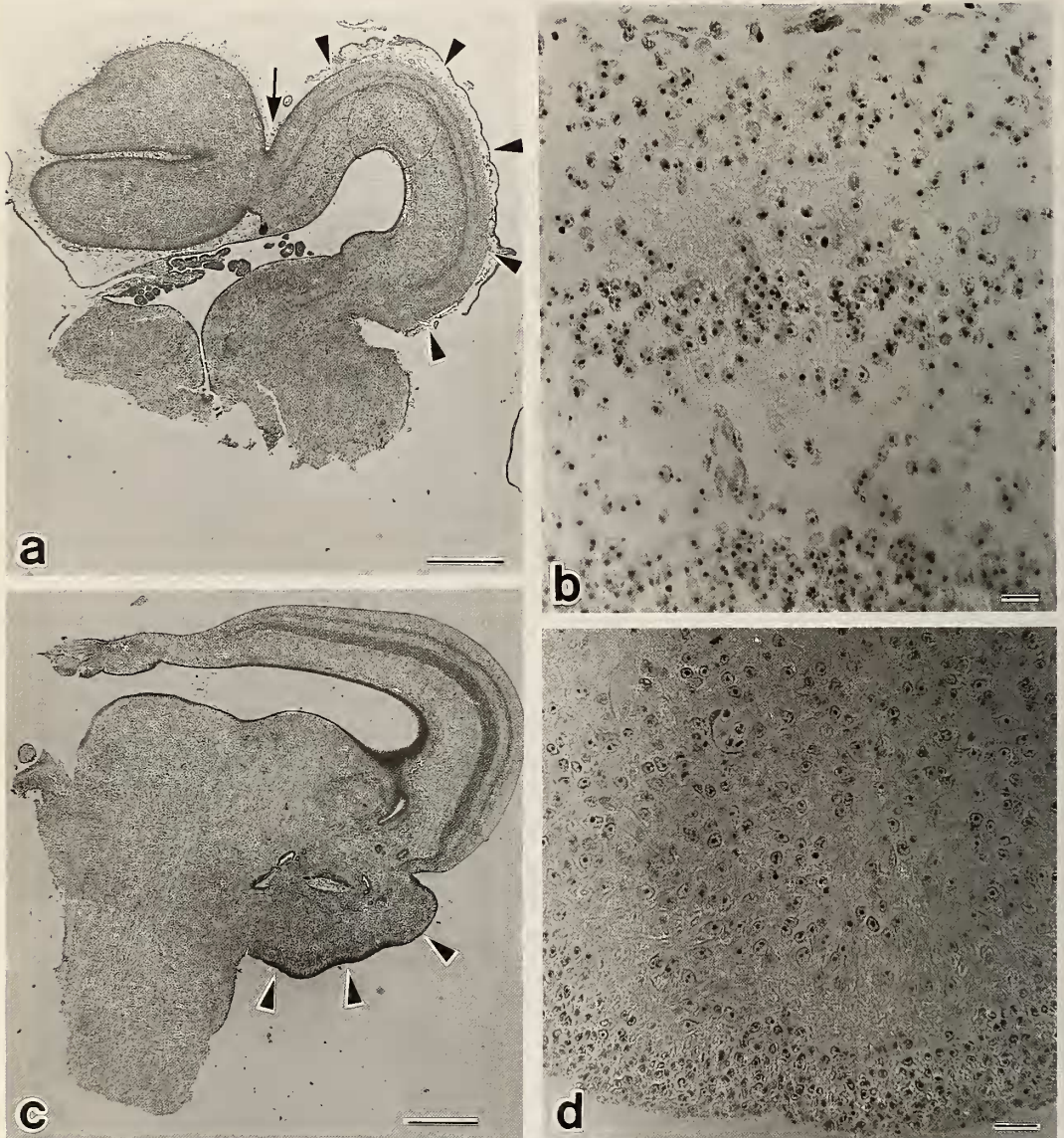
FIG. 3.   Transplantation of the mesencephalon into the metencephalon (a, b), and metencephalon into mesencephalon (c, d).
Transplantation was performed at around 10 somite stage. Alar plate of the mesencephalon transplanted into the metencephalon differentiated into the optic tectum (a, b). And alar plate of the metencephalon transplanted into the mesencephalon differentiated into cerebellar structure (c, d). Arrowheads in a and c show the transplants. Arrow in a shows the bondary between the host and the transplant. Bars in a and c: 500 μm, in b and d: 25 μm. (Taken from Nakamura [8])

tion within the necortex, not on the original position. This result supports the idea that tissue interaction plays an important role in the CNS development.

The alar plate of the prosencephalon transplanted into the metecephalon did not differentiate into the cerebellum. The results of heterotopic transplantations showed the limited capacity of the prosencephalon in differentiation. This indicates that determination occurs sequentially. Because the mode of morphogenesis of the optic tectum is different from that of the cerebellum, the tectum anlage is incapable of differentiating into the cerebellum.

It was shown that the alar plate of the mesencephalon differentiated into an ectopic optic tectum when transplanted into the prosencephalon or into the metencephalon [11, 8] (Fig. 3). The ectopic tectum differentiated between the telencephalon and the optic tectum proper, received retinal fibers. Alvarado-Mallart et al. [12] performed transplantations after dividing brain vesicles into rostral and caudal halves. Transplantations of the rostral part of the alar plate of the mesencephalon into the metencephalon showed that the transplants differentiated into the cerebellum. On the other hand, caudal part of the mesencephalon did not change their fate.

Nakamura [8] showed that metencephalon transplanted into the prosencephalon or into the mesencephalon kept its original fate, that is, the transplant differentiated into an ectopic cerebellum (Fig. 3). Similar results were obtained by Alvarado-Mallart et al. [12].

Recent report of Alvarado-Mallart et al. [12] showed that rostoral part of the metencephalon could differentiate into the optic tectum when transplanted into the mesencephalon but that it maintained its cerebellar structure at the diencephalon. It was also shown that caudal part of the metencephalon did not change its fate at the ectopic site. From these results they concluded that the rostral part of the brain vesicles has plasticity.

PLASTICITY OF THE ROSTRO-CAUDAL AXIS OF THE MESENCEPHALON

The mature retinotectal relationship is very strict. Temporal retinal ganglion cells project to the rostral part of the tectum, and nasal retinal ganglion cells project to the caudal part of the tectum [16]. Recent studies with a lipophilic fluorescent dye, DiI, have shown that axons from a tiny part of the temporal retina make tight focus of terminal arborization at the rostral part of the tectum [17]. It is an interesting question whether the polarity of the tectum is determined from an early stage of development. Rotation of tectum anlagen was performed [18, 19]. A quail tectum anlage was transplanted into a chick mesencephalon by rotating its rostrocaudal axis 180° at about 10 somite stage. On day 14 of incubation, a small crystal of DiI was placed at the temporal or rostral part of the retina on the contralateral side to the grafted tectum because the retina projects to the contralateral side of the tectum. With DiI, we can trace retinal fibers from a tiny part of the retina [20]. Embryos were fixed on day 16 of incubation and whole mounts of the retina and the tectum were observed under an epifluorescence microscope. After observations on the whole mounts, the specimens were embedded in paraffin, and cut serially. Feulgen and Rossenbeck staining [21] allows us to distinguish betwen quail and chick cells [1].

Eight complete and 10 partial chimeras were obtained; by complete, we mean that the tectum is etirely substituted by the transplant. In all the chimeras we obtained, rostrocaudal axis of the transplant was adjusted to that of the host, that is, temporal part of the retina projected to the rostral part of the tectum, though it was originally caudal, and the temporal part of the retina projected to the caudal part of the tectum (Fig. 4). The tecta made up of the quail graft were always smaller than those of the host (Fig. 5). This phenomenon was also noticed by Balaban et al. [22]. Senut and Alvarodo-Mallart [23] transplanted quail tectum anlagen homotopically into the chick embryo around 10 somite stage. During the normal course of ontogenesis, quail tectum differentiates faster than that of the chick [24]. Senut and Alvarado-
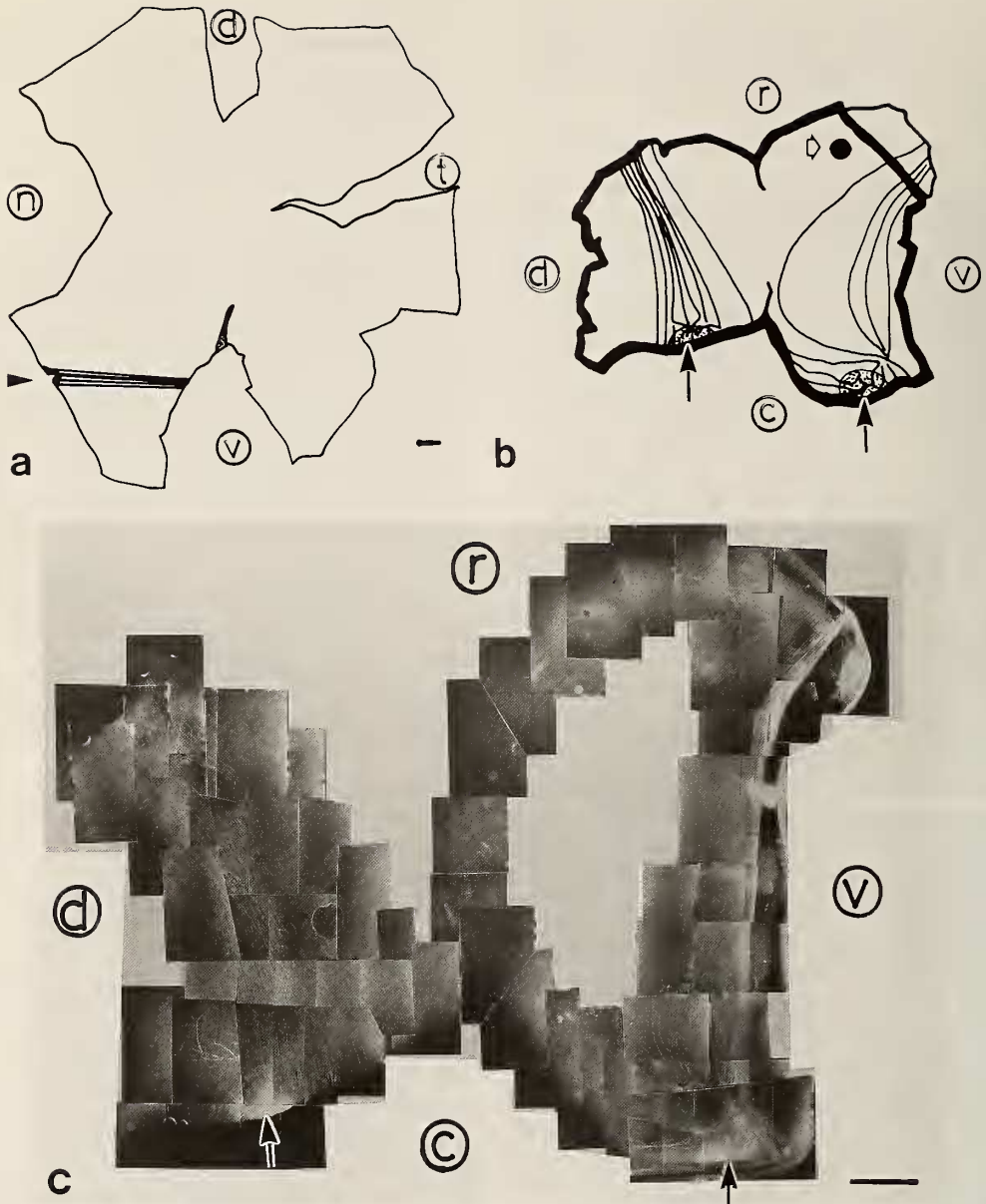
H. NAKAMURA

FIG. 4. Projection of nasal retinal ganlion cells to the caudal part of the tectum which was rotated of its rostrocaudal
axis through 180° at around 10 somite stage. (a) Camera lucida drawing of a whole mount of a retina. DiI was put
at the nasal part of the retina (arrowhead). n: nasal, d: dorsal, t: temporal, v: ventral. (b) Camera lucida drawing
of a whole mount of a rotated tectum shown in c.
Fibers from nasal part of the retina entered the contralateral tectum at the rostral part and extended to the caudal
pole of the tectum where the fibers made a tight focus of terminal arborization. r: rostral, v: ventral, c: caudal, d:
dorsal. Open arrow indicates a DiO crystal which was put at the caudal part of a mesencephalon of the transplant
(DiO crystal comes to the rostral part of the transplant after rotation). Solid arrows indicate the terminal zone
which is separated into 2 at the preparation of the whole mount specimen. The area encircled with thick line
shows the area of the transplant. (c) Whole mount of a rotated tectum.
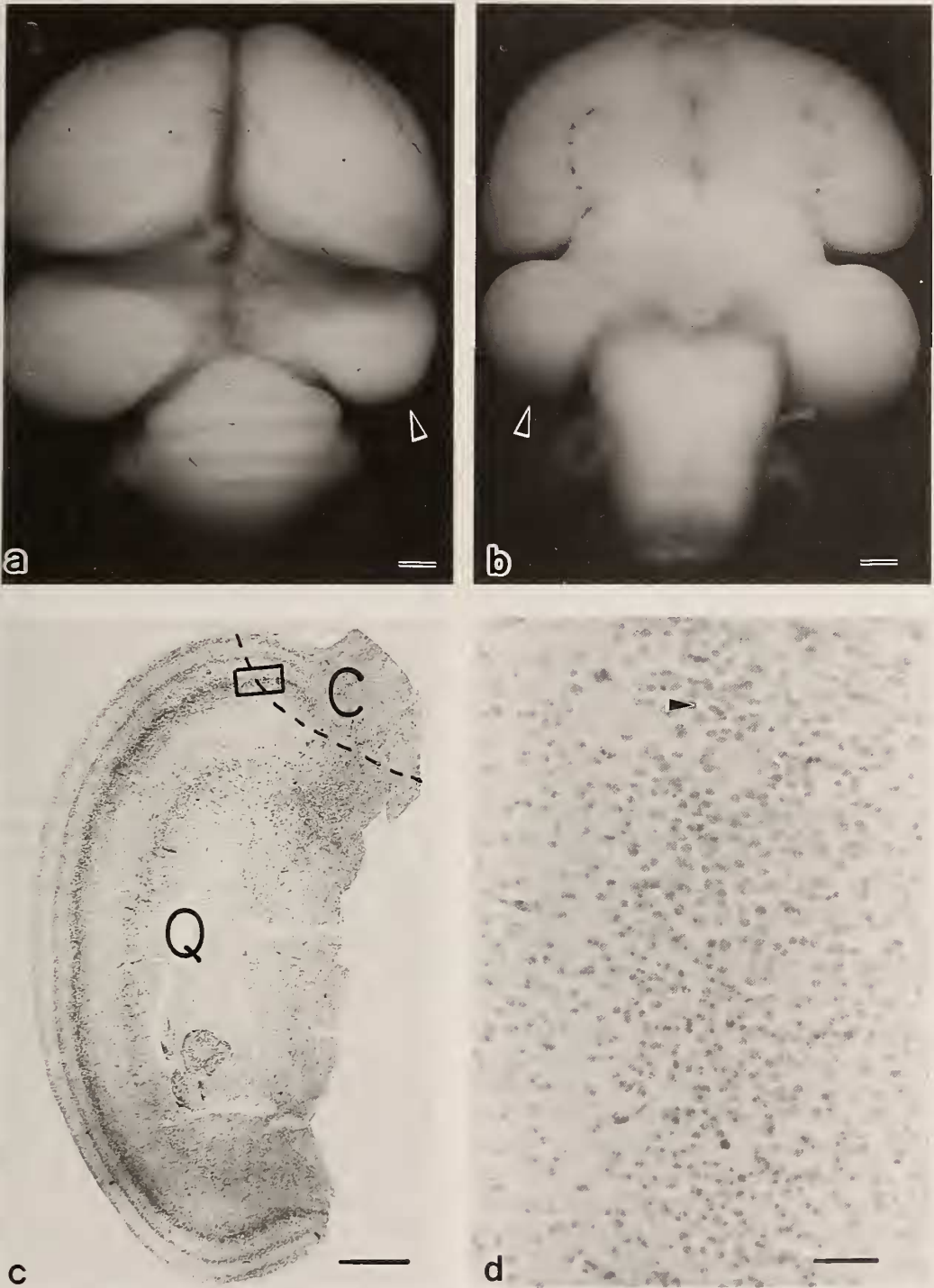Fiber trajectory and the terminal zone are shown in b. Bars: 1 mm. (Taken from Ichijo et al. [18])

FIG. 5. A chimeric brain in which tectum anlage was rotated of its rostrocaudal axis trough 180° at around 10 somite stage (The same specimen shown in Fig. 4). (a) Dorsal view, (b) ventral view, (c) A section cut parallel to the rostrocaudal axis of the tectum, Q: quail domain, C: chick domain, (d) High magnification at the boundary (arrowhead) of the transplant and the host. Bars in a, b, c: 1 mm, in d: 25 μm.

Transplantation was done at the right tectum (arrowhead in A, and B). The transplant (arrowhead) was always smaller than the host. (Taken from Ichijo et al. [18])

Mallart [23] found that the transplant (quail tissue) differentiated faster than contralateral host tectum (chick). They suggested that the schedule of the cytoarchitectonic development may be genetically determined, and may not be altered by epigenetic factors. Ichijo *et al.* [18] interpreted their result as that the speed of cytodifferentiation and the size of the tissue are determined genetically. Thus the size of the tectum which consists of quail cells may be always smaller than that of the host.

Histogenesis of the tectum after rotation of its rostrocaudal axis was studied by Matsuno *et al.* [25]. In the course of normal tectum development, rostral part differentiates faster than caudal part [26, 27]. Such developmental gradient across the rostrocaudal axis becomes discernible on day 5 of incubation. The transplant was compared with the quail tectum. Rostral part of the rotated tectum, though it had initially been caudal, had thicker wall of the tectum and neurogenesis was more advanced than in the caudal part. These are very interesting phenomena. On the one hand, the speed of the cytoarchitectonic differentiation is not adjusted beyond species. On the other hand, the speed of cytoarchitectonic differentiation is adjusted within the tissue.

EXPRESSION OF *HOMEOBOX* GENE IN THE TECTUM ANLAGEN

Recently, homeobox gene '*engrailed*' was reported to be expressed not only in *Drosophila* but also in a restricted segments of the vertebrate nervous system [28, 29]. In chick embryos, *en* gene is expressed in the anterior metencephalon to posterior mesencephalon. In the mesencephalon, caudal part strongly express *en* gene, and a gradient of *en* gene product arises along caudo-rostral direction. Martinez and Alvarodo-Mallart [30] rotated the tectum anlage at around 10 somite stage, and stained with a monoclonal antibody which specifically recognizes engrailed proteins. They found that the rostrocaudal specificity about *en* gene product was already regulated as that of the host after 20 hr os the tranplantation.

Rotation of the tectum anlagen gives consistent results. Retinotectal projection map was adjusted to that of the host [18]. Cytoarchitectonic develop-

ment of the tectum was also regulated and similar to that of the host (our unpublished observation). The transplant did not keep the original pattern of regional differentiaion. It has not yet been shown that homeobox gene '*engrailed*' is related to the establishment of the rostrocaudal specificity of the tectum, but the result that *en* gene expression is already regulated after 20 hr of transplantation indicates a possible role of *en* gene in the determination of the rostrocaudal axis of the tectum [29].

The results that *en* gene expression is regulated after 20 hr of tectum rotation and that subsequent rostrocaudal specificity is regulated conforming to the host pattern suggest that some environmental cues emanate from adjacent tissue. Since *en* gene is expressed strongly at the caudal part of the mesencephalon and there is a caudo-rostral gradient of *en* gene product, Alvarado-Mallart *et al.* [30] suggested that the metencephalon is responsible for regulatory signals on the rostrocaudal specificity of the optic tectum.

Other experiments imply that the diencephalon is responsible in determining the rostrocaudal specificity of the optic tectum. Chung and Cook [31] rotated the tectal primordia of *Xenopus* embryos. Rostrocaudal specificity was reversed only when ectopic diencephalon was developed caudally to the tectum, and was not reversed when ectopic diencephalon was not developed caudally. They proposed that the diencephalon controls the rostrocaudal specificity of the tectum. Further study is needed to elucidate axis determination of the tectum.

CONCLUSION

Study of the CNS development in quail-chick chimera is getting a fruitful results. The results suggest sequential determination in the CNS development and the importance of tissue interaction in the determination.

Optic tectum in birds is a visual center, and because of that, it has great advantage for experimental analysis. Retinotectal projection has long been a focus of studies, and much data have been accumulated. Recent studies with quail-chick chimera are adding important data. First, the

rostrocaudal axis of the tectum is not dtermined around 10 somite stage. When the rostrocaudal axis of the tectum anlage is rotated through 180°, the axis is adjusted to that of the host after 20 hr of transplantation. Later cytoarchitectonic differentiation and retinotectal map formation proceed similarly to those of the host tectum. It was recently shown that there is a rostrocaudal specificity of tectal membrane when the retinal axons enter the tectum [32]. Temporal retinal axons avoided the caudal tectal membrane and extended neurites on the rostral tectal membranes. Caudorostral gradient of the repulsive activity against temporal retinal fibers was also demonstrated [33]. These events may occur sequentially, the later event being induced by the former one. Thus, in the CNS development, morphogenesis and neural circuit formation may proceed interrelatedly.

ACKNOWLEDGMENTS

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